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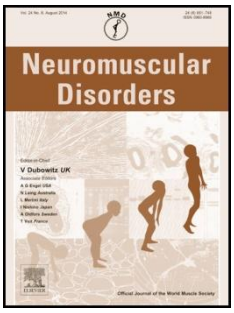

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Electromechanical delay components during skeletal muscle contraction and relaxation in patients with myotonic dystrophy type 1

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Short title: Electromechanical delays in DM1.

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Highlights

- Patients with myotonic dystrophy type 1 (DM1) were investigated isometrically
- We calculated the electromechanical delays during muscle contraction and relaxation
- Delays were partitioned into electrochemical and mechanical components
- Measurements reliability was very high in both patients and controls
- Delays were longer in DM1, especially during muscle relaxation

Abstract

The electromechanical delay during muscle contraction and relaxation can be partitioned into mainly electrochemical and mainly mechanical components by an EMG, mechanomyographic, and force combined approach. Components duration and measurements reliability were investigated during contraction and relaxation in a group of patients with myotonic dystrophy type 1 (DM1, n=13) and in healthy controls (n=13). EMG, mechanomyogram, and force were recorded in DM1 and in age- and body-matched controls from *tibialis anterior* (distal muscle*)* and *vastus lateralis* (proximal muscle) muscles during maximum voluntary and electrically-evoked isometric contractions. The electrochemical and mechanical components of the electromechanical delay during muscle contraction and relaxation were calculated off-line. Maximum strength was significantly lower in DM1 than in controls under both experimental conditions. All electrochemical and mechanical components were significantly longer in DM1 in both muscles. Measurements reliability was very high in both DM1 and controls. The high reliability of the measurements and the differences between DM1 patients and controls suggest that the EMG, mechanomyographic, and force combined approach could be utilized as a valid tool to assess the level of neuromuscular dysfunction in this pathology, and to follow the efficacy of pharmacological or non-pharmacological interventions.

Keywords: electromyogram; latency; mechanomyogram; MMG; muscle weakness; myotonia

Abbreviations

DM1: myotonic dystrophy type 1

EMG: electromyography

MMG: mechanomyography

F: force

SEC: series elastic components

$Delay_{TOT}$: total electromechanical delay during muscle contraction

Stim: stimulation current

$R\text{-}Delay_{TOT}$: total electromechanical delay during muscle relaxation

HC: healthy controls

S: experimental session

MVC: maximum voluntary contraction

TA: *tibialis anterior* muscle

VL: *vastus lateralis* muscle

pF: peak force

Δt: time interval during muscle contraction

$R\text{-}\Delta t$: time interval during muscle relaxation

RMS: root mean square

$MMG_{p\text{-}p}$: MMG peak-to-peak during muscle contraction

$R\text{-}MMG_{p\text{-}p}$: MMG peak-to-peak during muscle relaxation

1. Introduction

Myotonic dystrophy type 1 (DM1, OMIM 160900) is the most frequent form of inherited muscular dystrophy in adulthood, with a prevalence of about 1:8000 [1]. The adult-onset form is the most prevalent, with common clinical exacerbation in the second or third decade of life. Clinical manifestations of adult-onset DM1 involve a broad spectrum of systemic complications, such as cardiac conduction abnormalities and cardiomyopathy, cataract, central nervous system dysfunction, gastrointestinal symptoms, and endocrine abnormalities [1-4]. The main features at the skeletal muscle level are muscle weakness and grip and percussion myotonia [1]. Distal muscles are generally more compromised than the proximal ones [1, 5].

The mechanisms for muscle weakness, which involves difficulties to perform fine tasks with the hands, foot drop, and facial muscles ptosis, are not fully elucidated. Experimental evidence demonstrates that an alteration in the splicing of several proteins involved in Ca^{2+} homeostasis and in excitation-contraction coupling mechanisms may play a pivotal role [6-10]. Myotonia is characterised by a state of pathologically enhanced muscle excitability, in which involuntary trains of action potentials cause a delay in muscle relaxation after contraction [11]. This phenomenon has been associated to the alternative splicing of chloride channels at the muscle fibre level, which determines an alteration of membrane excitability [11]. Altogether, these skeletal muscle alterations in DM1 cause impairments in the cascade of the events involved in muscle contraction and relaxation.

Recently, a surface electromyographic (EMG), mechanomyographic (MMG) and force (F) combined approach has been proposed to get more insights on neuromuscular activation [12-16] and relaxation [15, 17, 18]. While surface EMG has been already widely used to monitor skeletal muscle electrical activity, MMG records and quantifies the low-frequency transverse oscillations propagating from the active muscle fibres to the skin surface during contraction (Orizio et al. 2003; Esposito et al. 2011a; Islamet al. 2013), thus representing the mechanical counterpart of EMG (Gordon & Holbourn, 1948). During muscle contraction, three main mechanisms contribute to

MMG generation, as shown in Fig. 1: (i) the gross lateral movement of the contracting fibres at the beginning of contraction (MMG complex), generated by the shortening of contractile elements before the slack of the elastic-connective tissue has been fully taken up, and F has been transmitted to the tendon insertion point [15]; (ii) the subsequent vibrations at the resonance frequency of the muscle (MMG ripple), reflecting the dimensional changes of the active fibres propagating towards the muscle surface [19-22]; and (iii) the gross lateral movement of the muscle at the end of contraction (R-MMG complex), due to the maximum acceleration of muscle surface caused by cross-bridges detachment and series elastic components (SEC) detensioning [23, 24].

During the on-phase of muscle contraction, the time lag between the onset of muscle electrical activation (EMG onset) and the beginning of F generation (F onset) has been traditionally defined as the electromechanical delay [25]. This time lag includes the electrochemical and mechanical events from motor unit action potential propagation at the sarcolemmal level to force transmission at the tendon insertion point. Similarly, a latency between the cessation of muscle electrical activation (end of EMG signal) and the beginning of F decay has been assessed during the relaxation phase [26], and defined as the relaxation electromechanical delay [27]. This latency spans from the cessation of sarcolemmal electrical activation to the cross-bridges and SEC return toward their pre-contraction status.

When also MMG is recorded, the three signals allow the partitioning of the total electromechanical delay ($Delay_{TOT}$) into (i) a mainly electrochemical component, which includes the events from the propagation of the motor unit action potential at the sarcolemmal level to myosin head rotation and pressure wave transmission to the skin surface [28, 29], and (ii) a mainly mechanical component, which reasonably provides a potential index of the time required for taking up the muscle-tendon unit slack, before F transmission becomes efficient at the tendon insertion point [12-14, 16]. When the muscle is electrically activated, the simultaneous recording also of the stimulation current (Stim) offers an additional $Delay_{TOT}$ component between Stim and EMG onset, related to the pre-synaptic and synaptic events [16].

At the end of a voluntary contraction, the total electromechanical delay during relaxation (R-$Delay_{TOT}$) can be partitioned with the addition of MMG in one mainly electrochemical and three consecutive mainly mechanical components. The first component includes the events from the cessation of the electrical activation of the sarcolemma and the beginning of Ca^{2+} reuptake in the sarcoplasmic reticulum to the transition of cross-bridges from force-generating to non-force-generating status, together with the pressure wave transmission to the skin surface. The second component comprises the beginning of the rapid change in sarcomere length and the increase in the detachment rate of cross-bridges. The third component incorporates the main phase of cross-bridge detachment and SEC relaxation. Lastly, the fourth component includes the time taken by the cross-bridges and SEC to return toward their pre-contraction status [15, 17, 18].

A similar approach, with the use of high frame rate ultrasound, has been recently proposed in patients with Duchenne dystrophy during contraction [30]. A lengthening of the overall delay due to mechanical component expansion was clearly observed.

In clinical settings, myotonia and muscle weakness are usually determined on patients with DM1 qualitatively or demi quantitatively by the Medical Research Council scale [31], by a dynamometer, and/or by physician's handgrip evaluation [32, 33]. Hence, a valid, non-invasive, and reliable tool to assess the degree of muscle dysfunction in DM1 could be of great interest for clinical trials involving new therapies.

Therefore, the aim of the study was twofold: (i) to assess the reliability and sensitivity of the measurement of the electromechanical delay components during both contraction and relaxation in patients with DM1; and (ii) to evaluate and discuss possible differences in delays' components duration between patients with DM1 and healthy controls (HC).

2. Materials and Methods

2.1. Participants

Patients with adult-onset DM1 were screened and selected for eligibility if: (i) aged between 18 and

70 yrs; (ii) with genetically confirmation of DM1 diagnosis; (iii) in presence of the myotonic phenomenon; (iv) without any antimyotonia therapy; (v) without cardiac pacemaker; (vi) without epilepsy; (vii) without concomitant neurological impairments or circulatory diseases at the lower limbs level. Thirteen patients with DM1 (age: 37 ± 14 yrs; body mass: 77 ± 13 kg; stature: 1.79 ± 0.08 m; age of onset: 28 ± 12 yrs; CTG triplet expansion: 431 ± 311; mean ± standard deviation (SD)) fulfilled all the inclusion criteria and participated to the study. Thirteen healthy age-matched male participants (age: 36 ± 8 yrs; body mass: 75 ± 9 kg; height: 1.80 ± 0.07 m; mean ± SD) volunteered as HC. After receiving a full explanation of the study aims and experimental procedures, patients with DM1 and HC signed an informed consent form. The study was approved by the local ethics committee and performed in accordance with the principles of the 1975 Declaration of Helsinki.

2.2. Experimental design

Patients with DM1 and HC reported to the laboratory two times. During the first visit, participants were familiarized with the experimental set-up and procedures. On a subsequent day, participants reported to the laboratory for a second visit for testing procedures, involving the assessment of maximum electrically evoked and voluntary strength and of inter- and intra-session reliability.

2.3. Experimental procedures

All experiments were carried out in a laboratory at a constant temperature of 22 ± 1 °C and relative humidity of 50 ± 5%. Participants were asked to abstain from caffeinated or other similar beverages in the 24 h preceding the tests, and to refrain from any heavy physical exercise with their lower limbs in the 48 h prior to the study.

During the first visit, participants were instructed to contract and fully relax the investigated muscle after contraction as quick as possible, with the aid of a visual feedback (F and EMG signals shown

on a computer screen). Then, participants underwent few trials of electrically evoked and voluntary contractions to determine their tolerance to electrical stimulation and their compliance to exert maximum efforts.

During the second visit, two experimental sessions (S_A and S_B, separated by 10 minutes of rest) to assess maximum electrically evoked and voluntary strength of the *tibialis anterior* (TA) and *vastus lateralis* (VL) muscles were performed. Participants seated on a purposely-made ergometer for F assessment of the lower limbs (Fig. 2), with an angle of 90 deg at the hip, knee, and ankle level. The peak electrically evoked F (pF) and maximum voluntary contraction (MVC) of the TA and VL were assessed in random order. Both pF and MVC were repeated three times, with 5 minutes of rest in between. During F assessment of TA, the dominant foot was attached firmly to a metal plate with a heel support by Velcro® straps (Velcro Industries Inc., Willemstad, Netherlands Antilles), while during F assessment of VL the distal third of the dominant leg was secured to a metal support by Velcro® straps (Fig. 2). The whole apparatus had a resonant frequency >200 Hz. The metal plate and the leg support were both connected to a calibrated load cell (mod SM-1000N and SM-2000N for ankle dorsiflexion and knee extension, respectively; operating linearly between 0 and 1000 N and between 0 and 2000 N, respectively; Interface, Crowthorne, UK) for the detection of F signal, which was then amplified (gain: x 200; mod. UM150, Biopac System, Santa Barbara, CA, USA) and successively driven to the auxiliary input of the EMG amplifier.

The surface EMG and MMG signals were detected during contraction and acquired by a multichannel amplifier together with Stim (mod. EMG-USB, OtBioelettronica, Turin, Italy; input impedance: >90 MΩ; CMRR: >96 dB; EMG and MMG bandwidth: 10-500 and 4-120 Hz, respectively; gain: x 1000 and x 2 for EMG and MMG, respectively), with a sampling rate of 10240 Hz. The EMG signal was detected by a linear array of four electrodes (mod. ELSCH004, OtBioelettronica, Turin, Italy; probe 45 mm x 20 mm; electrode length 2 mm; inter-electrode distance 10 mm) fixed to the skin by dual-adhesive foam (mod. AD004, OtBioelettronica, Turin,

Italy) and filled with conductive gel (Cogel, Comedical, Trento, Italy). The skin area under the EMG electrodes was cleaned with ethyl alcohol, abraded gently with fine sand paper and prepared with a conductive cream (Nuprep, Weaver and Co., Aurora, USA) to achieve an inter-electrode impedance below 2000 Ω. The third electrode of the EMG array was removed and replaced by a mono-directional accelerometer (mod. ADXL103, Analog Devices, Norwood, MA, USA; device weight: <1.0 g; sensitivity: 1000 mV/g; measure range: +/- 1.7 g) for MMG detection from the same muscle area as EMG.

For both muscles (TA and VL), the EMG array was placed over the muscle belly near the point of maximum skin displacement during contraction, along the direction of the muscle fibres, with the EMG electrodes positioned perpendicular to the major axes of the fibres between the tendon and the motor point, in accordance with the European recommendations for surface EMG [34].

Neuromuscular electrical stimulation was delivered to TA and VL in monopolar technique by an electrical stimulator (mod. St-Pro Multichannel Programmable Neuromuscular Stimulator, LiSin, Turin, Italy). For TA activation, the receiving electrode (130 x 100 mm) was positioned at the third distal of the leg, whereas the stimulating electrode (90 x 40 mm) was placed over the most proximal motor point of the muscle. For VL activation, the receiving electrode was positioned at the third proximal of the thigh and the stimulating electrode was placed over the most proximal motor point of the muscle. A set of brief 2-Hz stimulations of increasing amplitude was administered to determine the maximum compound motor unit stimulus. After the stimulus that elicited the maximal peak-to-peak M-wave was identified, participants rested for 5 min. Then, a set of three tetanic stimulations, consisting of a train of pulses (wave shape: biphasic; pulse duration: 304 μs; stimulation frequency: 50 Hz; current amplitude: 110% of the maximum compound motor unit stimulus; duration: 2s) was delivered. During stimulations, participants were instructed to maintain the muscles of the lower limb as relaxed as possible. The lack of activation of the antagonist muscles (*biceps femoris* and medial *gastrocnemius* muscles for VL and TA, respectively) was

monitored during contraction by an EMG linear array (mod. ELSCH004, 45 mm x 20 mm; electrode 2 mm x 1 mm; inter-electrode distance 10 mm; OtBioelettronica, Turin, Italy).

Measurements obtained from the three trials were used for intra-session reliability determination. To assess inter-session reliability, EMG, MMG and stimulation electrodes were removed after the first set of measurements (S_A) and repositioned after 10 minutes. Thereafter, all procedures were repeated again (S_B). Maps with some skin identification points (moles, angiomas, and scars) and with the position of the EMG-MMG integrated probe on the TA and VL were drawn on a transparency to allow repeated measurements from the same muscle area on different sessions.

2.4. Data analysis

The signals acquired (Stim, EMG, MMG, and F) were analysed off-line by a custom-built routine of a commercially available software (Labview 7.1. National Instruments, Austin, TX, USA).

2.4.1. EMG, MMG and F signals analysis.

Time domain analysis of the EMG signal allowed the calculation of the root mean square (RMS) from epochs of 1 s, corresponding to the central part of the F plateau reached during each contraction. From the MMG signal, the maximum displacement during the MMG (MMG_{p-p}) and R-MMG (R-MMG_{p-p}) complex were determined. The pF and MVC were assessed as the highest level of F achieved during the three trials of electrically evoked and voluntary contractions, respectively.

2.4.2. Delays calculation

Delays identification in a representative participant is given in Fig. 3. The criteria used to identify the reference points for delays components have been fully reported in previous investigations [14, 16, 18]. Briefly, during the electrically evoked contraction $Delay_{TOT}$ was partitioned into three distinct delays: (i) Δt Stim-EMG (mainly synaptic component), from the first positive peak of Stim

to EMG onset; (ii) Δt EMG-MMG (mainly electrochemical component), from the onset of EMG to the onset of MMG complex; and (iii) Δt MMG-F (mainly mechanical component) from MMG complex to F onset [16]. Under voluntary contraction, $Delay_{TOT}$ was divided into (i) Δt EMG-MMG (mainly electrochemical component), from EMG onset to MMG onset; and (ii) Δt MMG-F (mainly mechanical component), from MMG to F onset [14].

Under both electrically evoked and voluntary contractions, R-$Delay_{TOT}$ was partitioned into four components: (i) R-Δt EMG-F (mainly electrochemical component), spanning from EMG cessation to the beginning of F decay; (ii) R-Δt F-MMG_{p-p} (first mainly mechanical component), from the beginning of F decay to the beginning of the R-MMG complex; (iii) R-Δt MMG_{p-p} (second mainly mechanical component), from the beginning to the end of the R-MMG complex; and (iv) R-Δt MMG-F_{end} (third mainly mechanical component), from the end of the R-MMG complex to F return to baseline [18].

2.5. Statistical analysis

Raw data were analysed using a statistical software package (IBM SPSS Statistics v. 22, Armonk, NY, USA). To check the normal distribution of the sampling, a Shapiro-Wilk test was applied. Based on previous investigations by our group [35, 36] and on preliminary data, a sample size of 13 participants was selected to ensure a statistical power higher than 0.80, with a type 1 error <0.05. A one-way analysis of variance (ANOVA) for repeated measures was used on EMG, MMG and F values to determine possible differences among the three electrically evoked contractions and MVC within S_A and S_B. A Student's unpaired t-test was applied to evaluate differences between DM1 and HC in delays, EMG, MMG and F parameters. The magnitude of the changes was assessed using the effect size (ES) statistics. ES was classified as trivial for ES values <0.2, small between 0.2-0.6, moderate between 0.6-1.2, large between 1.2-2.0, and very large when >2.0 [37]. A two-way, mixed model Intraclass Correlation Coefficient (ICC) and the Standard Error of Measurements

calculation as a percentage (SEM%) were utilized to assess intra- and inter-session reliability. ICC values were considered as very high if >0.90, high if between 0.70 and 0.89, and moderate if between 0.50 and 0.69. The sensitivity in detecting the differences between the two experimental groups was checked by calculating the minimum detectable change at 95% confidence as a percentage (MDC_{95}%) [38]. The level of significance was set at α<0.05. Unless otherwise stated, the results are expressed as mean ± standard error (SE).

3. Results

None of the participants complained for any heavy discomfort or pain during testing procedures. During S_A and S_B, one-way ANOVA did not disclose significant differences among the three trials within each session for EMG, MMG and F parameters calculated in TA and VL during electrically evoked and voluntary contractions. The results were therefore pooled and used for further analysis.

3.1. Reliability and sensitivity of the measurements

ICC and SEM% for the investigated variables are reported in Tables 1-4 for HC and DM1. Intra- and inter-session reliability in HC resulted always from high to very high, with ICC ranging from 0.80 to 0.99. SEM% was between 1% and 7% of the relative mean value. Similarly, ICC in DM1 ranged from 0.79 to 0.99, with SEM% between 1% and 13%.

The MDC_{95}% calculated in HC and the percentage difference between HC and DM1 are presented in Table 5. All the variables showed relative differences between DM1 and HC higher than those required by MDC_{95}%.

3.2. EMG, MMG and F parameters

The differences between DM1 and HC in EMG, MMG and F parameters are presented in Fig 4.

EMG RMS (panel A) was significantly lower in DM1 than HC in both TA (p<0.05, ES ranging form -0.70 and -1.40) and VL (p<0.05, ES ranging form -0.58 and -1.15). $MMG_{p\text{-}p}$ and R-$MMG_{p\text{-}p}$

(panel B) were lower in DM1 compared to HC under both contraction regimes in TA (p<0.05, ES ranging from -0.69 and -1.49) and in VL (p<0.05, ES ranging from -0.69 and -1.49). pF and MVC (panel C) were always higher in HC than DM1 in TA (p<0.001, ES = -3.50 and -3.75 for pF and MVC, respectively), and VL (p<0.001, ES = -6.01 and -5.98 for pF and MVC, respectively).

3.3. Delays

The relative duration of $Delay_{TOT}$ and R-$Delay_{TOT}$ components during electrically evoked and voluntary contractions is reported in Fig. 5 and 6 for TA and VL, respectively. In both muscles, $Delay_{TOT}$ and R-$Delay_{TOT}$ components in DM1 were all significantly longer than in HC (p<0.05, ES for TA ranging from 0.98 to 3.55 and from 0.62 to 2.47 in electrically evoked and voluntary contractions, respectively; ES for VL ranging from 1.25 to 3.91 and from 1.24 and 3.52 in electrically evoked and voluntary contractions, respectively), with the only exception of Δt Stim-EMG in VL.

4. Discussion

The novel findings of the study were that: (i) intra- and inter-session reliability of delay components measurement in patients with DM1 was always from high to very high, with adequate levels of sensitivity; and (ii) all components of the delays in both the contraction and relaxation phases were longer in patients with DM1 than in HC. These results suggest that DM1 affected the duration of both the electrochemical and the mechanical events underpinning muscle contraction and relaxation. Noteworthy, given the very high level of reliability and sensitivity, this EMG, MMG, and F combined approach may be proposed as a valid means to investigate muscle contraction and relaxation characteristics in patients with DM1.

4.1. Reliability and sensitivity

The reliability analysis provided similar ICC values between patients with DM1 and HC. Reliability was always from high to very high in both groups, thus preparing the way to the applicability of this combined approach also to clinical settings.

In previous studies on HC under electrically evoked contraction, ICC values ranged from 0.919 to 0.989 and from 0.781 to 0.981 for intra- and inter-session reliability, respectively, for the delay components during the on-phase of muscle contraction [16]. Under voluntary contraction, only inter-session reliability analysis on delays components has been so far proposed, with ICC values ranging from 0.994 to 0.997 [14]. During relaxation after electrically evoked muscle activation, inter-session reliability provided ICC values ranging from 0.794 to 0.949 [17], while after voluntary activation only inter-day reliability was investigated, with ICC values ranging from 0.921 to 0.943 [18]. High levels of inter-operator reliability was also reported in a previous investigation, in which a similar analysis was conducted [16]. These previous findings are perfectly in line with those of the present investigation in HC (see Tables 1 and 2), thus clearly showing that this approach can provide reliable intra- and inter-session delay measurements also in patients with DM1. It must be noted, though, that previous studies investigated different skeletal muscles, such as the *biceps brachii* [14, 18] or *gastrocnemius medialis* [16, 17] muscles. Therefore, future studies in this direction to broaden delays reliability in different muscles may be required for both patients with DM1 and HC.

The difference in delays assessment between patients with DM1 and HC retrieved in the present investigation were larger than those requested by the $MDC_{95\%}$, thus indicating an adequate level of sensitivity. High intra- and inter-session reliability and sensitivity levels are two crucial requirements to introduce this approach in a clinical setting. Therefore, the present study represents a first solid step toward this direction.

4.2. EMG, MMG, and F parameters

In agreement with previous investigations [35, 36], maximum electrically evoked and voluntary strength were significantly lower in DM1 than in HC in both muscles. Lower EMG and MMG amplitude parameters values accompanied this force deficit in patients with DM1. Collectively, the DM1-induced alteration in Ca^{2+} release, the reduction in the number of efficient motor units, as witnessed by the lower EMG and MMG amplitude, and the increased fibrosis and adipose deposition may all concur to explain the reduction in force [5, 35, 36].

During relaxation, R-MMG_{p-p} was lower in DM1 by about 46% and 61% in TA and VL, respectively, compared to controls, indicating a slower maximum acceleration of the muscle surface toward its resting position. This finding well agrees with the myotonic phenomenon, which is typically retrieved in patients with DM1. Moreover, R-MMG_{p-p} was also suggested to correlate with Ca^{2+} reuptake [39]. Thus, an impairment also in this mechanism cannot be completely ruled out and may need further investigation.

4.3. Delays during muscle contraction

$Delay_{TOT}$ was longer in DM1 than in HC under both experimental conditions. With the only exception of Δt Stim-EMG during VL contraction, all the components contributed to the lengthening of $Delay_{TOT}$.

Δt Stim-EMG, reflecting the time lag between the origin of the action potential at the axonal level and the action potential propagation along the sarcolemma, thus including also the synaptic latency [16], was about 33% longer in DM1 than HC in TA. A longer duration of this component may suggest that an alteration of some electrochemical process involved in the synaptic latency may have occurred with DM1, at least in distal muscles, which are usually more affected than the proximal ones [1, 5].

The subsequent component, Δt EMG-MMG, includes events that are mainly electrochemical in nature, spanning from the propagation of the motor unit action potential at the sarcolemmal level to myosin head rotation, together with the pressure wave transmission to the skin surface detected by

MMG (Hufschmidt 1985; Petitjean et al. 1998). The longer Δt EMG-MMG in DM1 may be explained by the alteration in the splicing of several proteins, such as SERCA1 and CACNA1S, involved in Ca^{2+} homeostasis and in excitation-contraction coupling mechanism [6-10] thus possibly leading to a lengthened duration of the dihydropyridine and ryanodine receptors interaction, Ca^{2+} release by sarcoplasmic reticulum, and troponin activation [9, 10, 35].

Δt MMG-F is the $Delay_{TOT}$ component mainly reflecting the mechanical events involved in muscle contraction. This delay may provide a potential index of the time required for taking up the muscle–tendon unit slack, before F transmission becomes efficient at the tendon insertion point [12-14]. The longer duration of Δt MMG-F in DM1 may be suggestive of an alteration of the F transmission to the tendon insertion point. Likely, the modification occurring in DM1 at the muscle level, such as fibrosis and adipose deposition [5], may have reduced the whole efficiency in SEC tensioning.

4.4. Delays during muscle relaxation

R-$Delay_{TOT}$ was significantly longer in DM1 than HC by about 50%. Both the mainly electrochemical and the three mechanical components contributed to this difference between groups. As previously mentioned (see Introduction), myotonia consists in repetitive firing of some fibres due to an alternative splicing of chloride channels at the muscle fibre level that causes an increase in their membrane excitability [11]. This phenomenon leads to the inability of the muscle to fully relax in a certain period [11], and can play a pivotal role in explaining the lengthening of R-$Delay_{TOT}$.

R-Δt EMG-F, the mainly electrochemical component of R-$Delay_{TOT}$, likely includes the beginning of Ca^{2+} reuptake in the sarcoplasmic reticulum and the transition of cross-bridges from a force-generating to a non-force-generating status [17, 18, 40, 41]. The previously mentioned alteration in chloride channels excitability and in SERCA1 and CACNA1S splicing, may have delayed Ca^{2+} reuptake in the sarcoplasmic reticulum, [11], and in turn lengthened the mainly electrochemical R-$Delay_{TOT}$ component.

The myotonic phenomenon, which *per se* has an electrical origin, could provide a mechanistic insight also for the behaviour of the muscle-tendon unit during the mainly mechanical phases of muscle relaxation. Previous studies, indeed, focusing on HC, suggested that the mainly mechanical components of R-Delay$_{TOT}$ could reflect (i) the beginning of the rapid change in sarcomere length and the increase in the detachment rate of cross-bridges (R-Δt F-MMG$_{p\text{-}p}$); (ii) the main phase of the detachment of cross-bridges and SEC relaxation (R-Δt MMG$_{p\text{-}p}$); and (iii) the final return of cross-bridges and SEC to their pre-contraction status, respectively (R-Δt MMG-F$_{end}$) [17, 18]. This cascade of events is typical of a physiologic muscle relaxation. If an involuntary train of action potentials occurs during the last phases of muscle relaxation (i.e. when F has already began to decrease), the cross-bridges and SEC dynamics would be strongly affected by this phenomenon. This, in turn, would lead to an overall lengthening of the mainly mechanical components of R-Delay$_{TOT}$.

Some authors hypothesized two different mechanisms to describe the cross-bridges and SEC behaviour of a muscle presenting the myotonic phenomenon [5, 42]. The first mechanism implies two populations of muscle fibres: (i) normally relaxing fibres, and (ii) slowly relaxing fibres. As these two groups of fibres are relaxing in parallel, their forces sum, accounting for an abrupt transition between rapid and delayed relaxation. The normally relaxing fibres drive the initial relaxation phase, but once they have fully relaxed, the slowly relaxing fibres dominate and drive the terminal relaxation phase [5]. The second mechanism is related to SEC elastic properties [42]. Under isometric condition, SECs are stretched when myofilaments contract. The resultant elastic force, acting in a direction opposite to that of the myofilaments, is expected to be greatest during a maximum contraction, i.e., when cross-bridges are mostly engaged and can therefore overcome the initial **"**myotonic**"** forces during the first phase of muscle relaxation, when the cross-bridges begin to disengage. By contrast, elastic forces, which are attenuated when myofilaments return to their resting state, would yield to the **"**myotonic**"** forces in the last phase of relaxation [42].

Lastly, also the muscle fibres rearrangement in DM1 [1, 5], which includes basophilic regenerating fibres, splitting fibres, fibrosis and adipose deposition, may also be proposed as further mechanisms to explain the lengthening of the mainly mechanical components of R-Delay$_{TOT}$.

5. Conclusions

Our findings show that the EMG, MMG and F combined approach for Delay$_{TOT}$ and R-Delay$_{TOT}$ components provides reliable and sensitive measurements also in DM1 population. The differences between patients with DM1 and HC in Delay$_{TOT}$ and R-Delay$_{TOT}$ components, together with the high reliability levels, suggest that the proposed EMG, MMG and F combined approach may be used as a valid tool to assess the level of neuromuscular dysfunction in this pathology. This means may be used also to assess the degree of skeletal muscle impairment during the natural history of the disease, and to follow the efficacy of pharmacological (e.g., mexiletine or the upcoming trial with ISIS-DMPKrx) or non-pharmacological interventions in patients with DM1.

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Conflict of interest

No conflicts of interest to declare.

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Figure legends

Fig. 1 - Stim, EMG, MMG and F signals in a representative participant. Stimulation current (Stim), electromyographic (EMG), mechanomyographic (MMG), and force (F) signals during the on- (left sided grey bar) and off-phase (right sided grey bar) of muscle contraction.

Fig. 2 - Schematic representation of the experimental set-up. Positioning of the participant on the ergometer, of the EMG linear array electrodes, accelerometer and stimulation electrodes (stimulating and receiving electrodes), during trials on the *tibialis anterior* (panel A) and *vastus lateralis* (panel B) muscles.

Fig. 3 - Stim, EMG, MMG and F signals and delays in a representative participant. Contraction phase (left panel): The solid, dashed, dashed and dotted, and dotted lines indicate the onset of the stimulation current (Stim), electromyographic (EMG), mechanomyographic (MMG), and force (F) signals, respectively. Relaxation phase (right panel): The solid, dashed, dotted, short dashed and long dashed lines indicate the negative peak of EMG signal, the initial decrease in F signal, the beginning of the maximum displacement of the MMG signal ($MMG_{p\text{-}p}$), the duration of $MMG_{p\text{-}p}$ and the return of F signal at baseline, respectively.

Fig. 4 – Differences in EMG (panel A), MMG (panel B) and F parameters (panel C) in patients with DM1 compared to healthy controls (HC) for *tibialis anterior* and *vastus lateralis* muscles, during electrically evoked (left side column) and maximum voluntary contraction (right side column).

*p<0.05 DM1 *vs.* HC. RMS: Root mean square; MMG_{p-p}: MMG peak-to-peak during muscle contraction; R-MMG_{p-p}: MMG peak-to-peak during muscle relaxation; pF: peak force; MVC: maximum voluntary contraction.

Fig. 5 – $Delay_{TOT}$ and R-$Delay_{TOT}$ components duration in healthy controls (HC) and patient with DM1 in *tibialis anterior* muscle, during muscle contraction (upper panels) and relaxation (lower panels), and during electrically evoked (left side) and maximum voluntary contraction (right side). *p<0.05 DM1 *vs.* HC.

Fig. 6 - $Delay_{TOT}$ and R-$Delay_{TOT}$ components duration in healthy controls (HC) and patient with DM1 in *vastus lateralis* muscle, during muscle contraction (upper panels) and relaxation (lower panels), and during electrically evoked (left side) and maximum voluntary contraction (right side). *p<0.05 DM1 *vs.* HC.

Table 1: Intra- and inter-session (mean of the three trials in session A, S_A, and session B, S_B) reliability of $Delay_{TOT}$ and R-$Delay_{TOT}$ components in healthy controls for the *tibialis anterio*r muscle under electrically evoked and voluntary contractions. ICC: Intraclass correlation coefficient. SEM%: Standard Error of Measurements calculation as a percentage. Data are expressed as means ± standard deviation. For delays explanation, see Materials and Methods.

tibialis anterior m. Controls		Intra-session S_A				Intra-session S_B				Inter-session			
		1st trial	2nd trial	ICC	SEM%	1st trial	2nd trial	ICC	SEM%	S_A	S_B	ICC	SEM%
Electrically evoked contraction	Δt Stim-EMG (ms)	1.5±0.2	1.5±0.2	0.99	1	1.5±0.2	1.5±0.2	0.99	2	1.5±02	1.5±0.2	0.98	2
	Δt EMG-MMG (ms)	6.6.±0.5	6.8±0.7	0.80	4	6.8±0.7	6.7±0.5	0.82	4	6.7±0.7	6.8±0.6	0.80	4
	Δt MMG-F (ms)	13.6±1.3	13.8±1.3	0.91	3	14.2±1.7	13.8±1.5	0.89	6	13.7±1.3	14.0±1.6	0.89	5
	$Delay_{TOT}$ (ms)	21.7±1.5	22.1±1.4	0.92	2	22.5±2.1	22.0±2.3	0.90	3	21.9±1.4	22.3±2.2	0.90	3
	R-Δt EMG-F (ms)	19.9±2.1	20±2.1	0.99	3	19.9±2.2	20.3±2.2	0.91	3	19.8±2.2	20.2±2.2	0.94	3
	R-Δt F-MMG_{p-p} (ms)	56.0±6.5	56.1±7.4	0.90	4	55.3±5.9	57.0±4.1	0.88	6	56.1±7.0	56.2±5.0	0.88	5
	R-Δt MMG_{p-p}(ms)	105.4±12.1	104±9.5	0.90	3	109.9±11.9	106.7±10.8	0.83	7	104.7±10.8	108.3±11.4	0.85	5
	R-Δt MMG-F_{end} (ms)	67.4±4.5	68.1±10.5	0.87	4	67.7±5.7	67.2±4.6	0.93	4	67.8±7.5	67.5±5.2	0.89	4
	R-$Delay_{TOT}$ (ms)	248.4± 11.3	248.2±13.1	0.86	2	252.0±10.9	251.2±12.2	0.82	5	248.3±12.2	251.6±11.6	0.83	4
Voluntary contraction	Δt EMG-MMG (ms)	7.7±2.2	7.7±2.2	0.99	3	7.7±2.2	7.9±2.1	0.99	4	7.7±2.2	7.8±2.2	0.98	4
	Δt MMG-F (ms)	14.2±3.5	14.0±3.4	0.99	2	14.1±3.4	14.5±3.1	0.98	5	14.1±3.5	14.3±3.3	0.97	4
	$Delay_{TOT}$ (ms)	21.9±5.2	21.7±5.2	0.98	3	21.8±5.2	22.4±5.4	0.99	6	21.8±5.2	22.1±5.3	0.97	5
	R-Δt EMG-F (ms)	19.9±2.0	19.8±2.0	0.99	1	19.8±2.0	20.0±2.3	0.98	4	19.9±2.0	19.9±2.2	0.96	3
	R-Δt F-MMG_{p-p} (ms)	25.9±2.1	26.0±2.0	0.97	1	26.0±2.0	25.1±2.3	0.97	3	26.0±2.1	25.5±2.2	0.96	2
	R-Δt MMG_{p-p} (ms)	164.0±27.8	166.1±30.9	0.98	3	166.5±31.7	165.1±32.4	0.95	4	165.1±29.4	165.8±32.1	0.95	4
	R-Δt MMG-F_{end} (ms)	102.3±7.4	103.2±7.0	0.96	1	103.2±6.9	102.8±7.1	0.94	4	102.8±7.2	103.0±7.0	0.94	3
	R-$Delay_{TOT}$ (ms)	312.1±26.4	315.1±30.8	0.97	2	315.5±31.0	312.8±32.5	0.93	5	313.6±28.6	314.2±31.8	0.93	4

Table 2: Intra- and inter-session (mean of the three trials in session A, S_A, and session B, S_B) reliability of $Delay_{TOT}$ and R-$Delay_{TOT}$ components in healthy controls for the *vastus lateralis* muscle under electrically evoked and voluntary contractions. ICC: Intraclass correlation coefficient. SEM%: Standard Error of Measurements calculation as a percentage. Data are expressed as means ± standard deviation. For delays explanation, see Materials and Methods.

***vastus lateralis* m. Controls**		**Intra-session S_A**				**Intra-session S_B**				**Inter-session**			
		1st trial	2nd trial	ICC	SEM%	1st trial	2nd trial	ICC	SEM%	S_A	S_B	ICC	SEM%
Electrically evoked contraction	Δt Stim-EMG (ms)	1.5±0.2	1.5±0.3	0.99	2	1.5±0.2	1.5±0.3	0.99	2	1.5±0.3	1.5±0.2	0.96	3
	Δt EMG-MMG (ms)	7.7±1.2	7.7±1.4	0.97	3	7.6±1.4	7.6±1.4	0.93	4	7.7±1.3	7.6±1.4	0.92	5
	Δt MMG-F (ms)	12.1±1.6	12.4±2.0	0.91	5	13.0±2.4	12.1±1.9	0.87	6	12.3±1.8	12.5±2.1	0.87	6
	$Delay_{TOT}$ (ms)	21.3±2.7	21.6±3.2	0.94	3	22.1±3.6	21.2±3.1	0.93	4	21.5±3.0	21.6±3.3	0.91	5
	R-Δt EMG-F (ms)	19.7±0.9	19.8±1.1	0.98	2	20.2±.01	19.6±1.0	0.97	3	19.8±1.0	19.8±1.1	0.96	3
	R-Δt F-MMG_{p-p} (ms)	52.0±9.5	52.6±9.7	0.95	4	52.9±10.2	51.7±10.0	0.93	5	52.3±9.6	52.3±10.1	0.91	6
	R-Δt MMG_{p-p} (ms)	75.4±9.3	76.2±11.0	0.91	4	78.1±7.5	74.9±10.5	0.89	5	75.8±10.2	76.5±9.0	0.88	6
	R-Δt MMG-F_{end} (ms)	78.2±15.0	78.2±15.0	0.99	2	78.3±15.0	77.2±15.6	0.93	3	78.2±15.0	77.8±15.3	0.93	3
	R-$Delay_{TOT}$ (ms)	225.3±26.6	226.5±27.3	0.97	2	229.5±25.5	223.3±28.0	0.95	4	225.9±27.0	226.4±26.7	0.93	5
Voluntary contraction	Δt EMG-MMG (ms)	7.6±1.6	7.6±1.6	0.99	2	7.5±1.7	7.5±1.7	0.99	3	7.6±1.6	7.5±1.7	0.96	4
	Δt MMG-F (ms)	13.8±1.9	13.9±2.1	0.97	3	14.1±2.0	13.7±2.1	0.95	5	13.9±2.0	13.9±2.0	0.93	4
	$Delay_{TOT}$ (ms)	21.4±2.2	21.5±2.4	0.99	2	21.6±2.3	21.2±2.4	0.98	2	21.5±2.3	21.4±2.3	0.96	3
	R-Δt EMG-F (ms)	17.2±3.0	17.1±3.0	0.99	2	17.0±2.9	16.9±3.1	0.98	4	17.2±3.0	17.0±3.0	0.96	5
	R-Δt F-MMG_{p-p} (ms)	22.9±3.4	23.0±3.5	0.97	3	23.0±3.5	22.7±3.6	0.97	3	23.0±3.5	22.8±3.5	0.94	6
	R-Δt MMG_{p-p} (ms)	142.0±24.0	140.3±23.4	0.97	3	139.9±23.3	139.4±24.6	0.94	4	141.2±23.7	139.7±24.0	0.93	6
	R-Δt MMG-F_{end} (ms)	97.1±17.9	97.2±18.1	0.98	3	96.1±18.1	96.0±18.7	0.94	4	97.2±18.0	96.0±18.4	0.93	5
	R-$Delay_{TOT}$ (ms)	279.2±32.1	277.6±31.8	0.96	2	276.0±31.7	275.0±33.2	0.91	5	278.4±32.0	275.5±32.4	0.91	6

Table 3: Intra- and inter-session (mean of the three trials in session A, S_A, and session B, S_B) reliability of $Delay_{TOT}$ and R-$Delay_{TOT}$ components in patients with DM1 for the *tibialis anterior* muscle under electrically evoked and voluntary contractions. ICC: Intraclass correlation coefficient. SEM%: Standard Error of Measurements calculation as a percentage. Data are expressed as means ± standard deviation. For delays explanation, see Materials and Methods.

***tibialis anterior* m. DM1**		**Intra-session S_A**				**Intra-session S_B**				**Inter-session**			
		1st trial	2nd trial	ICC	SEM%	1st trial	2nd trial	ICC	SEM%	S_A	S_B	ICC	SEM%
Electrically evoked contraction	Δt Stim-EMG (ms)	2.0±0.6	2.0±0.6	0.98	4	2.1±0.5	2.0±0.6	0.97	5	2.0±0.6	2.1±0.6	0.96	5
	Δt EMG-MMG (ms)	13.3±3.8	13.2±5.3	0.96	8	12.6±6.6	13.6±4.6	0.94	9	13.3±4.6	13.1±5.6	0.91	10
	Δt MMG-F (ms)	18.5±7.4	18.5±7.0	0.90	11	19.9±8.6	18.9±7.3	0.89	12	18.5±7.2	19.4±8.0	0.87	13
	$Delay_{TOT}$ (ms)	33.8±8.5	33.7±9.1	0.92	9	34.6±12.9	34.6±9.0	0.89	10	33.8±8.8	34.6±10.9	0.86	11
	R-Δt EMG-F (ms)	31.3±3.2	31±3.7	0.98	4	31.3±3.7	31.9±3.5	0.97	4	31.2±3.4	31.6±3.6	0.96	5
	R-Δt F-MMG_{p-p} (ms)	76.2±20.6	76.2±18.3	0.98	3	77.2±19.9	77.5±19.8	0.97	4	56.2±19.5	57.4±19.9	0.96	4
	R-Δt MMG_{p-p} (ms)	166.6±78	167.0±63.1	0.96	6	172.7±81.8	170.8±71.9	0.93	8	166.8±70.6	171.7±76.9	0.89	9
	R-Δt MMG-F_{end} (ms)	406.2±145.1	406.7±112.2	0.95	5	396.6±109.0	416.1±131.1	0.88	8	406.5±128.7	406.4±120.1	0.81	10
	R-$Delay_{TOT}$ (ms)	680.3±159.4	680.9±194.3	0.92	4	677.8±177.1	696.3±180.3	0.89	6	680.6±176.9	687.1±178.7	0.85	8
Voluntary contraction	Δt EMG-MMG (ms)	11.1±2.5	11.2±2.9	0.94	6	11.0±2.5	11.4±2.8	0.94	6	11.2±2.7	11.2±2.6	0.94	5
	Δt MMG-F (ms)	30.0±10.1	30.0±10.0	0.98	5	29.7±10.3	30.7±10.2	0.97	6	30.0±10.1	30.2±10.3	0.95	6
	$Delay_{TOT}$ (ms)	41.1±11.9	41.2±12.1	0.97	5	40.7±12.1	42.1±12.2	0.96	6	41.2±12.0	41.4±12.2	0.94	7
	R-Δt EMG-F (ms)	28.0±4.1	28.2±5.2	0.91	4	27.0±4.2	28.8±4.7	0.90	6	28.1±4.7	27.9±4.5	0.89	8
	R-Δt F-MMG_{p-p} (ms)	34.7±7.1	35.6±6.5	0.91	6	34.8±6.5	36.0±6.9	0.92	6	35.2±6.8	35.4±6.7	0.93	5
	R-Δt MMG_{p-p} (ms)	196.8±60.4	209.3±94.2	0.89	10	211.0±100.0	207.9±78.8	0.88	11	203.1±77.3	209.4±89.4	0.87	11
	R-Δt MMG-F_{end} (ms)	386.6±109.0	386.2±145.1	0.97	5	396.7±112.2	395.6±129.5	0.88	7	386.4±127.1	396.2±120.9	0.79	8
	R-$Delay_{TOT}$ (ms)	646.1±170.4	659.3±211.5	0.98	4	659.5±219.5	668.3±194.7	0.91	6	652.7±191.0	663.9±207.1	0.83	7

Table 4: Intra- and inter-session (mean of the three trials in session A, S_A, and session B, S_B) reliability of $Delay_{TOT}$ and R-$Delay_{TOT}$ components in patients with DM1 for the *vastus lateralis* muscle under electrically evoked and voluntary contractions. ICC: Intraclass correlation coefficient. SEM%: Standard Error of Measurements calculation as a percentage. Data are expressed as means ± standard deviation. For delays explanation, see Materials and Methods.

***vastus lateralis* m. DM1**		**Intra-session S_A**				**Intra-session S_B**				**Inter-session**			
		1st trial	2nd trial	ICC	SEM%	1st trial	2nd trial	ICC	SEM%	S_A	S_B	ICC	SEM%
Electrically-evoked contraction	Δt Stim-EMG (ms)	1.7±0.4	1.7±0.4	0.84	9	1.6±04	1.7±0.4	0.83	10	1.7±0.4	1.6±0.4	0.81	11
	Δt EMG-MMG (ms)	10.0±2.3	10.3±2.6	0.97	4	10.4±2.7	10.2±2.5	0.95	5	10.2±2.5	10.3±2.6	0.93	6
	Δt MMG-F (ms)	15.9±2.6	15.9±2.7	0.93	3	14.8±2.9	15.5±2.7	0.89	5	15.9±2.7	15.2±2.8	0.84	7
	$Delay_{TOT}$ (ms)	27.6±4.1	27.9±4.1	0.93	4	26.8±4.9	27.4±4.4	0.90	5	27.8±4.1	27.1±4.6	0.86	6
	R-Δt EMG-F (ms)	22.0±1.9	22.3±2.0	0.95	2	22.6±2.1	22.3±2.0	0.94	3	22.2±1.9	22.4±2.0	0.92	3
	R-Δt F-MMG_{p-p} (ms)	73.8±11.9	74.2±12.3	0.93	4	75.4±13.1	74.5±12.4	0.92	5	74.0±12.1	74.9±12.8	0.91	5
	R-Δt MMG_{p-p} (ms)	146.7±36.7	154.3±44.7	0.93	6	141.7±35.0	147.6±38.8	0.91	7	150.5±40.7	144.6±36.9	0.89	8
	R-Δt MMG-F_{end} (ms)	323.8±92.2	316.8±84.1	0.95	6	312.1±91.4	317.6±89.2	0.93	7	320.3±88.2	314.8±90.3	0.90	8
	R-$Delay_{TOT}$ (ms)	566.3±111.3	567.6±99.0	0.97	3	551.8±109.6	561.9±106.6	0.90	7	567.0±105.2	556.9±108.1	0.82	10
Voluntary contraction	Δt EMG-MMG (ms)	9.6±0.5	9.6±0.6	0.96	1	9.6±0.6	9.6±0.6	0.96	1	9.6±0.6	9.6±0.6	0.95	1
	Δt MMG-F (ms)	28.2±5.9	28.0±6.2	0.91	6	28.5±6.0	28.2±6.0	0.90	7	28.1±6.1	28.4±6.0	0.89	7
	$Delay_{TOT}$ (ms)	37.8±6.2	37.6±6.5	0.91	5	38.1±6.3	37.8±6.3	0.91	6	37.7±6.4	38.0±6.3	0.90	6
	R-Δt EMG-F (ms)	22.4±1.7	22.5±2.2	0.95	3	21.6±1.6	22.2±1.8	0.93	4	22.5±2.0	21.9±1.7	0.90	5
	R-Δt F-MMG_{p-p} (ms)	32.6±1.3	32.5±1.6	0.94	2	32.7±1.6	32.6±1.5	0.94	3	32.6±1.5	32.7±1.6	0.94	3
	R-Δt MMG_{p-p} (ms)	186.5±41.6	186.3±40.5	0.97	5	181.6±48.1	184.8±43.4	0.94	6	186.4±41.1	183.2±45.8	0.91	7
	R-Δt MMG-F_{end} (ms)	299.1±107.5	300.1±106.4	0.99	4	297.2±116.8	298.8±110.2	0.96	5	299.6±107.0	298.0±113.5	0.93	6
	R-$Delay_{TOT}$ (ms)	540.6±136.2	541.4±135.3	0.99	3	533.1±152.5	538.4±141.3	0.96	5	541.0±135.8	535.7±146.9	0.92	7

Table 5: $MDC_{95\%}$ (data of session A, S_A, and session B, S_B, were pooled for controls) and percentage difference in $Delay_{TOT}$ and R-$Delay_{TOT}$ components in patients with DM1 compared to healthy controls in the *tibialis anterior* and *vastus lateralis* muscles, under electrically evoked and voluntary contraction. $MDC_{95\%}$, minimum detectable change at 95% confidence as a percentage. Δ%: percentage difference between DM1 and controls. Data are expressed as means ± standard deviation. For delays explanation, see Materials and Methods.

		Tibialis anterior m.				Vastus lateralis m.			
		Controls	DM1	$MDC_{95\%}$	Δ%	Controls	DM1	$MDC_{95\%}$	Δ%
Electrically evoked contraction	Δt Stim-EMG (ms)	1.5±0.2	2.1±0.6	4	37	1.5±0.3	1.7±0.4	4	10
	Δt EMG-MMG (ms)	6.8±0.7	13.2±5.1	8	96	7.7±1.4	10.3±2.6	7	34
	Δt MMG-F (ms)	13.9±1.5	19.0±7.6	9	37	12.4±2.0	15.6±2.8	12	25
	$Delay_{TOT}$ (ms)	22.1±1.8	34.2±9.9	5	55	21.6±3.2	27.5±4.4	8	27
	R-Δt EMG-F (ms)	20±2.2	31.5±3.5	6	58	19.9±1.0	22.4±2.1	6	13
	R-Δt F-MMG_{p-p} (ms)	56.2±6.0	76.8±19.7	9	37	52.3±9.9	74.5±12.5	9	42
	R-Δt MMG_{p-p} (ms)	106.5±11.1	169.3±73.8	8	59	76.2±9.6	147.6±38.8	8	94
	R-Δt MMG-F_{end} (ms)	67.7±6.4	406.5±124.4	9	501	78.0±15.2	317.6±89.3	7	307
	R-$Delay_{TOT}$ (ms)	250.0±11.9	678.8±177.8	7	172	226.2±26.9	562.0±106.7	8	148
Voluntary contraction	Δt EMG-MMG (ms)	7.8±2.2	11.2±2.7	7	45	7.6±1.7	9.6±0.6	6	27
	Δt MMG-F (ms)	14.2±3.4	30.1±10.2	8	112	13.9±2.0	28.3±6.1	8	103
	$Delay_{TOT}$ (ms)	22.0±5.3	41.3±12.1	9	88	21.5±2.3	37.9±6.4	3	76
	R-Δt EMG-F (ms)	19.9±2.1	28.0±4.6	8	41	17.1±3.0	22.2±1.9	7	30
	R-Δt F-MMG_{p-p} (ms)	25.8±2.2	35.3±6.8	6	37	22.9±3.5	32.7±1.6	6	43
	R-Δt MMG_{p-p} (ms)	165.5±30.8	206.3±83.4	7	25	140.5±23.9	184.8±43.5	8	32
	R-Δt MMG-F_{end} (ms)	102.9±7.1	391.3±124.0	7	280	96.6±18.2	298.8±110.3	7	209
	R-$Delay_{TOT}$ (ms)	313.9±30.2	658.3±199.1	7	110	277.0±32.2	538.4±141.4	7	94